

(19)

Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 0 382 583 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
17.01.1996 Bulletin 1996/03

(51) Int. Cl.⁶: C07D 255/02, C07D 257/02

(21) Application number: 90301478.5

(22) Date of filing: 12.02.1990

(54) Aza macrocycles and processes for their preparation

Aza-Macrozyklen und Verfahren zu deren Herstellung

Azamacrocycles et procédé pour leur préparation

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(30) Priority: 10.02.1989 GB 8903024
10.02.1989 GB 8903025

(43) Date of publication of application:
16.08.1990 Bulletin 1990/33

(73) Proprietor: CELLTECH THERAPEUTICS LIMITED
Slough, Berkshire SL1 4EN (GB)

(72) Inventors:
• Parker, David
Durham DH1 4DG (GB)
• Millican, Thomas Andrew
Maidenhead Berkshire SL6 0dy (GB)
• Beeley, Nigel Robert Arnold
Oxfordshire OX9 3LQ (GB)

(74) Representative: Hallybone, Huw George et al
London WC1A 2RA (GB)

(56) References cited:

EP-A- 0 305 320	WO-A-86/02352
WO-A-89/01475	WO-A-89/01476
DE-A- 2 447 279	DE-A- 3 713 842

- CHEMICAL ABSTRACTS, vol. 97, no. 24, December 13, 1982, Columbus, Ohio, USA; BRYDEN C.C. et al.: "Multinuclear NMR study of three aqueous lanthanide shift reagents: complexes with EDTA and two macrocyclic ligands", page 617, column 2, abstract-no. 206 960z & Rare Earths Mod. Sci. Technol. 1982, 3, 53-7
- CHEMICAL ABSTRACTS, vol. 109, no. 18, October 31, 1988, Columbus, Ohio, USA; FUKUDA Y. et al.: "1,4,7-triazacyclononane-N,N',N''-trialkanoic acids-metal complexes and electrochromic elements using them", page 765, column 1, abstract-no. 160 661p & Jpn. Kokai Tokkyo Koho JP 62,298,578 (87,298,578)

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 0 382 583 B1

Description

Field of the Invention

This invention relates to functionalised aza macrocycles, to metal complexes thereof, to conjugate compounds containing the functionalised aza macrocycles and metal complexes thereof and to their use in diagnosis and therapy.

Background to the Invention

The attachment of metal ions to proteins, peptides and other, smaller molecules is a fast expanding technology, which has numerous proven and potential applications in research, in industry and, particularly, in medicine.

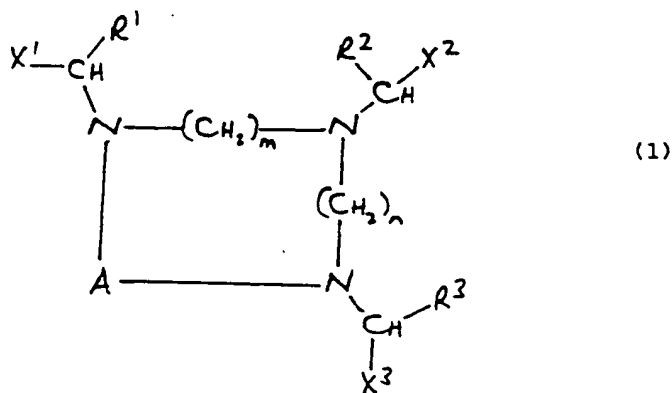
In recent years, much of the impetus behind the development of this technology has been the ability to link metal ions to antibodies, especially monoclonal antibodies. Such metal labelled antibodies have found a widespread use, especially in medicine, where they have been employed, for example, to target the metal ion to a specific tissue type, both *in vitro* and *in vivo*. Thus, metal labelled antibodies have applications in locating specific tissue types (e.g. employing computer-aided tomographic techniques where the metal ion is in some way detectable) and in the treatment of cell disorders (e.g. treating mammalian tumours where the metal ion is a cytotoxic radionuclide).

Conventionally, attachment of the metal ion to a protein such as an antibody has been achieved by complexation by an acyclic chelate such as a substituted diethylenetriaminepentaacetic acid [Gansow O. A. *et al.* Inorg. Chem., (1986), 25, 2772] or ethylenediaminetetraacetic acid [Meares, C. F. *et al.* Acc. Chem. Res., (1984), 17, 202] covalently linked to the antibody. Such acyclic complexes however tend to be unstable *in vivo* either as a result of acid-catalysed decomplexation or competitive chelate binding by Ca^{2+} or Zn^{2+} in serum, or as a result of competition from transferrin [Moerlein, S. M. *et al.* Int. J. Nuc. Med. Biol., (1981) 8, 277]. The lack of stability can result in uncomplexed metal atoms in the body which have a cytotoxic effect on healthy tissue (e.g. bone marrow) or which markedly reduce the signal-to-noise ratio of an imaging technique.

A possible alternative to the use of acyclic chelates in the labelling of antibodies is the use of macrocyclic ligands, which has been suggested by a number of workers [Gansow O. A. *et al.* Am. Chem. Soc. Symp. Ser., (1984), 241, 215; UK Patent Specification Publication No. 2122641; International Patent Specifications Nos. WO89/01475 and WO89/01476 and European Patent Specification No. 305320; and Moi M. K. *et al.* Anal. Biochem., (1985), 148, 249-253].

We have now found a new class of functionalised aza macrocycles, members of which are able to form kinetically inert complexes with metal ions. The macrocycles of the invention are particularly useful for attachment to proteins, especially antibodies, to provide conjugate compounds capable of binding metals to give complexes which are advantageously stable *in vivo*.

Thus, according to one aspect of the present invention we provide a compound of general formula (1);



wherein

A is a group $-(\text{CH}_2)_p-$ or $-(\text{CH}_2)_p \text{N(R)}(\text{CH}_2)_q-$ where R is a group $-\text{CH(R}^4)\text{X}^4$;
m, n, p, and q is each an integer 2;

R^1 , R^2 , R^3 and R^4 , which may be the same or different, is each a group $-\text{CO}_2\text{H}$, $-\text{P(R}^5)\text{O}_2\text{H}$ (where R^5 is a hydrogen atom or a C_{1-6} alkyl or alkoxy group), $-\text{PO}_3\text{H}_2$ or $-\text{CONR}^6\text{R}^7$ (where R^6 and R^7 , which may be the same or different is each a hydrogen atom or a C_{1-6} alkyl group);

X¹, X², X³ and X⁴, which may be the same or different, is each a hydrogen atom or a C₁₋₆ alkyl group, or a linker group of the formula -L-Z wherein L and Z are as defined below, with the proviso that at least one of X¹, X², X³ or X⁴ is a linker group; and metal complexes and/or salts thereof.

It will be appreciated that formula (1) [and, where appropriate, the following formulae herein], is intended to cover all stereoisomers of the compounds concerned, including mixtures thereof.

In the compounds of formula (1), it will be appreciated that the nature of the linker group may be varied widely without substantially affecting the usefulness of the compounds.

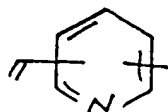
In the following definition, and in the same context whenever it appears below, the term "interrupted by" as applied to cycloaliphatic or aromatic groups is to be understood to also mean that these particular groups may additionally be present linked to the terminal carbon atom of the hydrocarbonyl chain represented by L, at the opposite end of the chain to the carbon atom attached to the macrocycle.

L is an optionally substituted straight or branched C₁₋₂₀alkylene, C₂₋₂₀alkenylene, or C₂₋₂₀alkynylene chain, optionally interrupted by one or more -O- or -S- atoms or C₅₋₈cycloalkylene (e.g. cyclopentylene or cyclohexylene), C₆₋₁₂aromatic (e.g. phenylene or substituted phenylene), C₅₋₁₀heteroaromatic (e.g. furanyl, pyridyl), -N(R⁸)-, -CON(R⁸)- or -N(R⁸)CO- groups (where R⁸ is a hydrogen atom or a C₁₋₆ alkyl group).

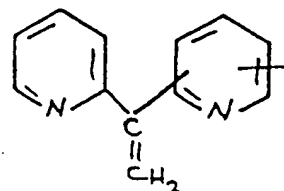
Examples of substituents which may be present on the chain L include halogen atoms, e.g. fluorine, chlorine, bromine, or iodine atoms or groups selected from C₁₋₆alkoxy (e.g. methoxy or ethoxy), hydroxy, nitro, -N(R⁹)(R¹⁰), [where R⁹ is a hydrogen atom or a C₁₋₆alkyl group and R¹⁰ is a C₁₋₆alkyl group; e.g. -NHCH₃ or -N(CH₃)₂], or substituted amido, e.g. a group of formula -(CH₂)_dCON(R¹¹)(R¹²) [where d is zero or an integer 1 to 4 inclusive, R¹¹ is a hydrogen atom or a C₁₋₆alkyl group, e.g. methyl and R¹² is an optionally substituted C₁₋₆alkyl group].

Substituted alkyl groups represented by R¹¹ include for example C₁₋₆alkyl groups substituted by one or more halogen atoms, or nitro, amino or hydroxy groups.

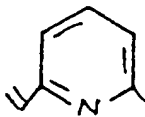
Z is a halogen atom, for example a chlorine, bromine or iodine atom, or a group selected from OH, -SH, -NH₂, hydrazine (-NHNH₂), or a derivative thereof, [for example -N(CH₃)NH₂, -NHCONHNH₂, -NHCSNHNH₂, or phenyl hydrazine], -NCO, -NCS, -COR¹³, [where R¹³ is a halogen atom such as a chlorine or bromine atom, or a N₃, C₁₋₆alkoxy, e.g. methoxy, C₆₋₁₂aryloxy (e.g. nitrophenyloxy or dinitrophenyloxy), imidyloxy (e.g. succinimidyloxy) or imidazolyloxy group], imide, e.g. maleimide, a vinyl group of formula -Het¹-C(Het²)=CH₂ (where Het¹ and Het², which may be the same or different, is each a nitrogen containing heterocyclic group, e.g. a pyridyl group or Het¹ is a nitrogen containing heterocyclic group and Het² is a hydrogen atom), for example a vinyl pyridyl group of formula



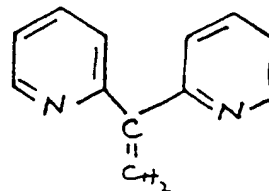
or



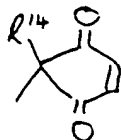
especially



or



or a dione of formula



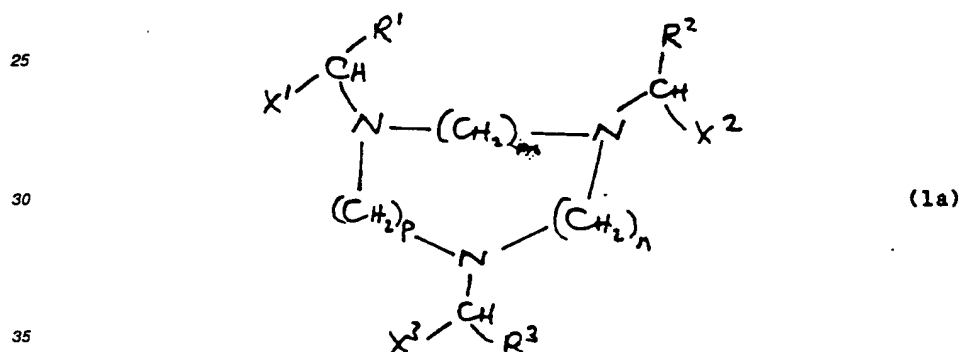
Metal complexes of the compounds of formula (1) in which A is $-(CH_2)_p N(R)(CH_2)_q-$ include complexes wherein the metal is di- or tripisitive and has a coordination number 6 or greater, especially 8. Examples of such metals include indium (In), copper (Cu), lead (Pb), bismuth (Bi), yttrium (Y), terbium (Tb), gallium (Ga), gadolinium (Gd) and scandium (Sc). In, Y, Ga, Tb, Gd, and Sc are preferred, particularly In, Y, Gd, and Ga. In general the metal is preferably a radioactive isotope. Yttrium, especially ^{90}Y , is particularly preferred.

Metal complexes of the compounds of formula (1) in which A is $-(CH_2)_p-$ include complexes wherein the metal is di- or tri-positive and has a coordination number from 2 up to 6, especially 6. Examples of such metal(s) include indium (In), copper (Cu), lead (Pb), bismuth (Bi), cobalt (Co) and gallium (Ga). In, Ga, Co and Cu are preferred, particularly In and Ga. In general the metal is preferably a radioactive isotope. Indium, especially ^{111}In , is particularly preferred.

15 Salts of the compounds of formula (1) include salts with bases, e.g. sodium or potassium salts, or acid addition salts such as hydrobromides or hydrochlorides. Pharmaceutically acceptable salts are particularly preferred.

A further preferred group of compounds of formula (1) wherein A is $-(CH_2)_p-$ or $-(CH_2)_pN(R)(CH_2)_q-$ is that wherein one or two of X^1 , X^2 , X^3 and X^4 is a linker group and the remainder are alkyl groups or, especially, hydrogen atoms.

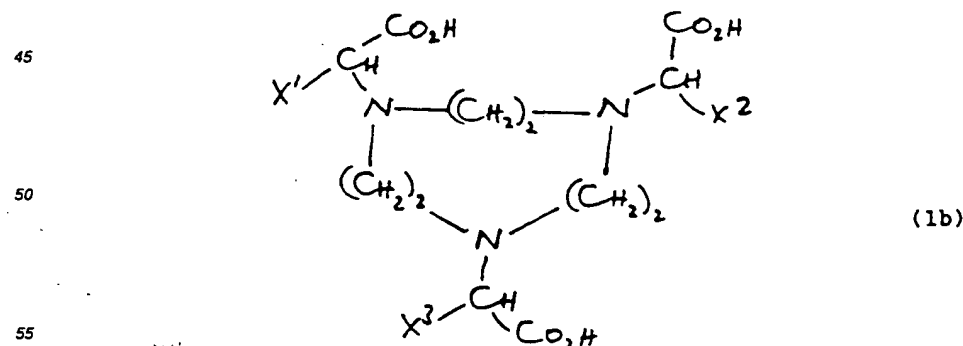
One group of compounds of formula (1) has the formula (1a):



wherein m, n, p, R¹, R², R³, X¹, X² and X³ are as defined for formula (1); and metal complexes and/or salts thereof.

40 Indium complexes of the compounds of formula (1a) are particularly preferred.

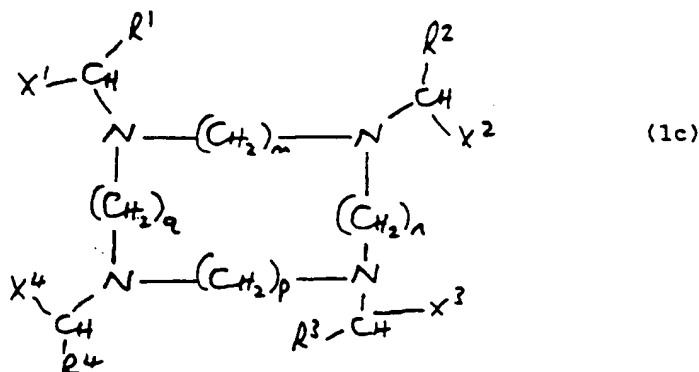
Particularly important compounds of formula (1a) are those of formula (1b)



wherein one or two of X¹, X², or X³ is a linker group and the remainder are hydrogen atoms; and metal complexes and/or salts thereof.

Indium complexes of the compounds of formula (1b) are particularly preferred.

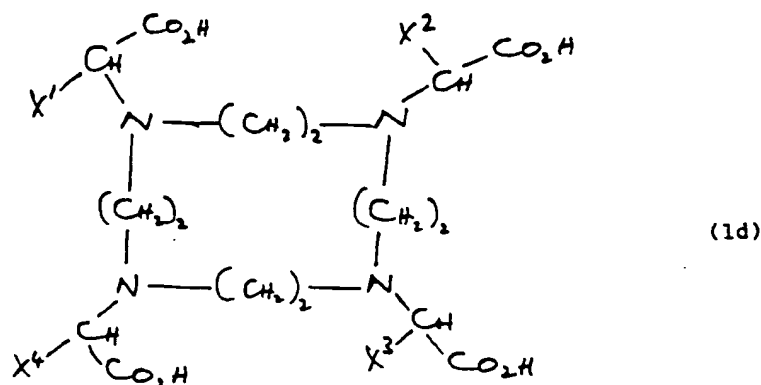
Another group of compounds of formula (1) has the formula (1c):



wherein m, n, p, q, R¹, R², R³, R⁴, X¹, X², X³ and X⁴ are as defined for formula (1); and metal complexes and/or salts thereof.

Yttrium complexes of the compounds of formula (1c) are particularly preferred.

An important group of compounds of formula (1c) are those of formula (1d)



wherein one or two of X¹, X², X³ or X⁴ is a linker group and the remainder are hydrogen atoms; and metal complexes and/or salts thereof.

Yttrium complexes of the compounds of formula (1d) are particularly preferred.

The compounds of formula (1) and the metal complexes and/or salts thereof have a diagnostic use as imaging agents *in vitro* and *in vivo*. The compounds of formula (1) and the metal complexes and/or salts thereof are also cytotoxic agents and may be used in the treatment of abnormal cell disorders, for example in the treatment of tumours. For use as diagnostic and/or therapeutic agents, the compounds of formula (1) may be employed using conventional methods, (e.g. for formulation and presentation) already in use for metal complexing agents.

For application of the compounds of formula (1) as imaging or cytotoxic agents, it is generally preferable to couple the compounds to other molecules such as proteins, especially antibodies, peptides or carbohydrates to form conjugate compounds, and the compounds of formula (1) are particularly well adapted for use in this respect.

Thus, according to a further aspect of the invention, we provide a conjugate compound which comprises a compound of formula (1), or a metal complex and/or salt thereof, coupled to a protein, peptide or carbohydrate.

The compound of formula (1) may be coupled through any thiol, amino, carboxyl, hydroxyl, aldehyde, aromatic or heteroaromatic group present in the protein, peptide or carbohydrate.

In a preferred aspect of the invention, we provide a conjugate compound which comprises a compound of formula (1) or a metal complex and/or salt thereof, coupled to an antibody.

It is to be understood that conjugate compounds according to the invention may contain more than one molecule of a compound of formula (1) couple to any one protein, peptide or carbohydrate molecule.

Particularly useful conjugate compounds according to the invention are those comprising a compound of formula (1b) or formula (1d) or a metal complex and/or salt thereof, coupled to an antibody. The indium and yttrium complexes of these conjugates are especially important.

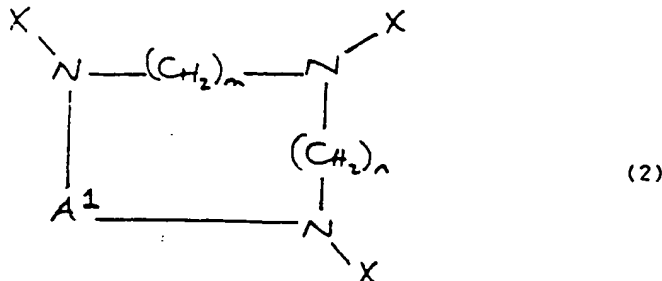
The compounds of formula (1) and conjugate compounds of the invention may be formulated for use in accordance with conventional practice. Thus according to a further aspect of the invention we provide a composition comprising a compound of formula (1); or a conjugate compound comprising a compound of formula (1) coupled to a protein, peptide or carbohydrate, or a metal complex and/or salt thereof, together with one or more pharmaceutically acceptable carriers.

Particularly suitable compositions according to the invention are those adapted for parenteral administration, especially by injection or infusion. Suitable formulations of this type include suspensions solutions or emulsions of the compound or conjugate in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Alternatively the compound or conjugate may be in powder form for reconstitution with a suitable vehicle, e.g. sterile pyrogen-free water before use. If desired the compound or conjugate may be presented in unit dosage form, and/or together with one or more active ingredients or imaging agents. Suitable formulations of this type include solutions of the compounds of formula (1) in isotonic saline.

The quantities of compounds of formula (1) used in formulations according to the invention will vary according to the intended cell target, but may be easily determined in accordance with conventional practice for reagents of this type.

Compounds of the invention may be prepared by the following processes wherein the groups and symbols R^1 , R^2 , R^3 , R^4 , m , n , p , q , X^1 , X^2 , X^3 and X^4 are as defined for formula (1) except where stated otherwise. Where a metal complex is desired as a final product, the complexation with a metal atom may be carried out as a final step in the production process, as described below for the complexation of compounds of formulae (1), or alternatively it may be desirable to complex the metal at an earlier stage in the process, providing of course that the requisite macrocycle structure is present. In the following processes, it may be desirable to use starting materials in which functional groups in the linker group are in a protected state, or which contain a precursor of the group, as discussed below.

Thus according to a further aspect of the invention a compound of formula (1) wherein at least one of X^1 , X^2 , X^3 and X^4 is other than a linker group, or a metal salt thereof may be prepared by reaction of a compound of formula (2):



[wherein A^1 is $-(CH_2)_p-$ or $-(CH_2)_p N(H) (CH_2)_q-$ a least one of the groups X is a hydrogen atom and the remainder is each a group $-CH(R^1)X^1$, where R^1 is as previously defined and X^1 is a linker group] with a compound $DCH(R^2)X^2$ (where X^2 is as defined for formula (1), but is not a linker group, and D is a displaceable group, for example a halogen atom such as a bromine atom) followed, where necessary, by removal of any protecting group.

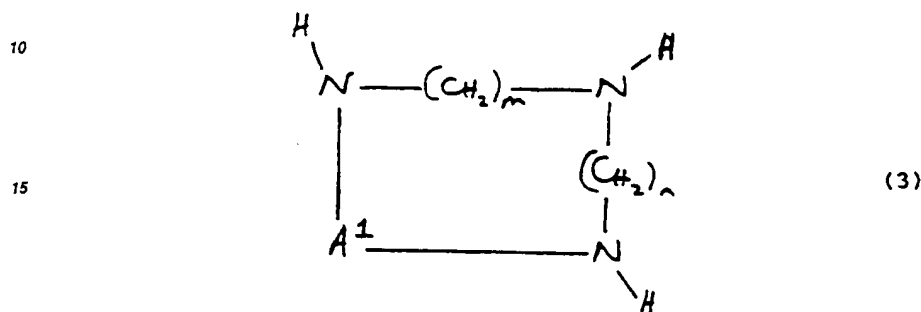
The reaction may be performed in a solvent such as water or an organic solvent such as a nitrile, e.g. acetonitrile, or an alcohol, e.g. ethanol; or an amide, e.g. dimethylformamide, in the presence of a base such as an alkali metal carbonate or hydroxide, e.g. sodium, potassium or caesium carbonate, or sodium, potassium or lithium hydroxide, at an elevated temperature e.g. the reflux temperature.

Where metal complexes of formula (1) are required (or any other suitable macrocyclic intermediate described herein) these may be prepared by treating the compound with a metal salt (for example a metal halide) in an appropriate solvent for example an aqueous or non aqueous solvent, (e.g. acetonitrile, acetone, propylene carbonate, dimethylformamide or dimethylsulphoxide) at any suitable temperature from 0°C to 100°C such as 10°C to 80°C e.g. around 60°C .

A conjugate compound according to the invention may be prepared by reaction of a compound of formula (1) or a metal complex thereof with a protein, peptide or carbohydrate in a aqueous solvent, for example an inorganic buffer such as a phosphate buffer at an appropriate temperature.

Salts of compounds of formula (1) and their metal complexes may be prepared by conventional means, for example by reaction with an appropriate base or acid in a suitable aqueous solvent.

Intermediates of formula (2) may be prepared by reaction of a compound of formula (3)



with a compound $DCH(R^1)X^1$ (where X^1 is a linker group or a protected derivative thereof) in the presence of a base in a suitable solvent at an elevated temperature as just described for the preparation of compounds of formula (1). By varying the molar ratio of the compound of formula (2) and the compound $DCH(R^1)X^1$ such that the latter is increased relative to the former, (for example from around 2:1 to 1:1 and further) compounds of formula (2) containing more than one $-CH(R^1)X^1$ group as just defined may be prepared.

Intermediates of formula (3) are either known compounds or may be prepared from known starting materials using methods analogous to those used for the preparation of the known compounds.

The following Examples illustrate the invention.

Example 1

35 (a) To a stirred solution of 1,4,7,10-tetraazacyclododecane (0.057g) in dry dimethylformamide (5ml) was added anhydrous potassium carbonate (0.05g). To this mixture at 80°C under nitrogen was added 2-bromo-6-benzamidoethyl hexanoate (0.113g) in dimethylformamide (5ml) over 2 hours. HPLC analysis revealed that after 2 days reactions were essentially complete to give, after removal of dimethylformamide a single major product [CM-300; gradient elution; t=0: A=80%, B=0%, C=20%; t=5min: A=60%, B=20%, C=20%; t=10 min: A=0%, B=80%, C=20% (with A=H₂O, B=1M ammonium acetate (pH 5.6), C=CH₃CN) flow rate = 1.4ml: min⁻¹]. Retention time = 7.5min. m/e (CI) 433 (M⁺+1) δ_H (CDCl₃) 1.25 (3H, t, CO₂CH₂CH₃), 1.45-1.79 (6H, mult, CH₂CH₂CH₂CH₂NH), 2.39-3.13 (16H m, ring CH₂'s) 3.32-3.50 (3H, m, CONHCH₂ and OCOCH), 4.14 (2H, q, CH₂O), 7.37-7.50 (4H, m, arom plus NHCO), 7.79 (2H, d+d ortho arom H).

40 (b) The compound prepared in part (a) was not isolated but was redissolved in dimethylformamide (5ml). To this solution was added dry potassium carbonate (0.15g) and at 80°C ethyl bromoacetate (0.165g) was added to a solution in dimethylformamide (2ml) over 15 min. Reaction was complete in 4 hours [HPLC analysis gave a major product at t=6.19 min under the conditions defined in part (a)]. After removal of dimethylformamide *in vacuo* the residue was chromatographed on neutral alumina (0.5% methanol/CH₂Cl₂ - 5% methanol/CH₂Cl₂) to yield as a pale oil the compound of formula (1) wherein A is $-(CH_2)_pN(R)(CH_2)_q-$, m, n, p and q is each an integer 2; R¹, R², R³ and R⁴ is each a group $-CO_2CH_2CH_3$; X¹, X³ and X⁴ is each a hydrogen atom; and X² is a group $-(CH_2)_4NHCOPh$ (where Ph is phenyl). m/e (DCE, ethanol) 692 (M⁺+1). δ_H (CDCl₃) 1.25 (12H, t+t+t, Me), 1.40-1.90 (6H, mult, CH₂CH₂CH₂CH₂NHCO), 2.13-3.20 (16H, mult, ring CH₂), 3.30-3.55 (9H, mult, CH₂CO+CHCO+CH₂NHCO), 4.08-4.18 (8H, mult., OCH₂) 7.40 (3H, brd, arom H), 7.94-8.07 (3H, brd + mult., ortho CH + NHCO).

55 Example 2

A solution of the compound of Example 1(b) (30mg) in hydrochloric acid (6M, 10ml) was heated to reflux for 36h. After cooling, and washing with diethyl ether (3 x 3ml), the removal of solvent under high vacuum yielded the compound of formula (1) wherein A is $-(CH_2)_pN(R)(CH_2)_q-$, m, n, p and q is each an integer 2, R¹, R², R³ and R⁴ is each a group -

CO_2H , X^1 , X^3 and X^4 is each a hydrogen atom and X^2 is a group $-(\text{CH}_2)_4\text{NH}_2^-$ as its tetrahydrochloride (28mg). $\delta_{\text{H}}(\text{D}_2\text{O})$ 1.64 (6H, br. mult., CH_2C), 2.93-4.16 (25H, mult. br., $\text{CH}_2\text{N} + \text{CHN}$), m/e (negative FAB, m-nitrobenzyl alcohol) 476 (M^-), 475 ($\text{M}^- - 1$).

5 Example 3

To a solution of the compound of Example 2 (9.5mg) in dry DMF (200 μl) was added a solution of N-succinimidyl-3-maleimidopropionate (14.0mg) in dry DMF (60 μl), and N-methyl morpholine (40mg) yielding a slight precipitate. Addition of MilliQ water (0.003ml) dissolved the precipitate and the mixture was held at 30°C for 24h. After removal of solvent under reduced pressure, the residue was purified on reverse-phase HPLC (Spherisorb 50DS2) to yield as a colourless glass and compound of formula (1) corresponding to the compound of Example 2 except that X^2 is a group $-(\text{CH}_2)_4\text{NHCO}(\text{CH}_2)_2-$ m/e (FAB, m-nitrobenzylalcohol) 628 ($\text{M}^+ + 1$), 552, 493, 409. $\delta_{\text{H}}(\text{D}_2\text{O})$ 6.85 (2H, s, $\text{CH}=\text{CH}$), 3.96-3.91 (7H, mult., $\text{CH}_2\text{N} + \text{CHN}$), 3.85 3.05 (20H mult. br. $\text{CH}_2\text{N} + \text{CH}_2\text{NHCO} + \text{CH}_2\text{NCO}$), 2.50 (2H, t, $\text{J}=6.4$, CH_2CONH), 1.65 (6H, mult., CH_2C). Rt = 8.9 min (Spherisorb ODS2, 1.4ml min $^{-1}$, t=0, 95% 0.1% TFA in H_2O , 5% 0.1% TFA in CH_3CN t=20, 5% 0.1% TFA in H_2O , 95% 0.1% TFA in CH_3CN).

To a solution of the tetraacid (10 μmol) in ammonium acetate buffer (90 μl , pH6.0) was added 5 μl of a ^{90}Y solution (2 μCi) and the mixture was incubated for 0.5h at 37°C. Reaction was quenched by addition of a 20 fold excess of diethylenetriaminedipentaacetic acid (DTPA) and the relative amounts of ^{90}Y complex and ^{90}Y -DTPA determined by HPLC radiometry (AX-300 anion exchange: eluant 0.2M NH_4OAc , 10% CH_3CN). A radiolabelling yield of 77% was calculated. In the presence of a 500 fold excess of DTPA at 25°C the relative concentration of the radiolabelled complex was measured at 24, 48 and 72h and seen not to diminish ($\pm 2\%$).

Example 4

To a solution of 1,4,7-triazacyclononane (0.104g) in dry dimethylformamide (5ml) was added potassium carbonate (0.117g) followed by 2-bromo-6-benzamidoethyl hexanoate (0.276g) as a solution in dimethylformamide (3ml). After stirring for 50h at 50°C, the cooled solution was filtered and solvent removed under reduced pressure to give a colourless oil, m/e (DCI, ethanol) 652 ($\text{M}^+ + 1$); HPLC [CM300; 2 major peaks (λ_{max} 235nm) at 2.4 and 4.7 min with t=0: A=70%, B=10%, C=20%; t=10min: A=0%, B=80%, C=20% (A= H_2O , B=1M ammonium acetate, C= CH_3CN)].

To a solution of the crude oil in dry ethanol (2ml) was added caesium carbonate (0.163g) and ethyl bromoacetate (0.083g). The mixture was stirred for 20 hours at 60°C when the solution was cooled, filtered and solvent removed to yield a pale yellow residue which was chromatographed on neutral alumina (1% methanol/ CH_2Cl_2) to give as a colourless oil [Rf=0.5 (2.5% methanol/ CH_2Cl_2)] the compound of formula (1) wherein A is $-(\text{CH}_2)_p$, m, n and p is each an integer 2, R^1 , R^2 and R^3 is each a group $-\text{CO}_2\text{CH}_2\text{CH}_3$, X^1 and X^2 is each a group $-(\text{CH}_2)_4\text{NHCOPh}$ (where Ph is phenyl) and X^3 is a hydrogen atom. m/e (DCI, ethanol) 738 ($\text{M}^+ + 1$), 621, 420. $\delta_{\text{H}}(\text{CDCl}_3$ 298K) 1.24 (9H, t, CH_3 , $\text{J}=7.1\text{Hz}$), 1.29-1.68 (12H, mult., $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCO}$), 2.77-3.46 (20H, mult., CH_2N , CHN), 4.11 (6H, q + q, CH_2O), 6.66 (2H, brs, NHCO), 7.42 (6H, mult., arom H), 7.79 (4H, dd, ortho CH).

40 Example 5

The compound of formula (1) wherein A is $-(\text{CH}_2)_p$, m, n and p is each an integer 2, R^1 , R^2 and R^3 is each a group $-\text{CO}_2\text{CH}_2\text{CH}_3$, X^1 is a group $-(\text{CH}_2)_4\text{NHCOPh}$ (where Ph is phenyl) and X^2 and X^3 is each a hydrogen atom was prepared using the methods of Example 4 except 1,4,7-triazacyclononane and 2-bromo-6-benzamidoethyl hexanoate were used in a 2:1 molar ratio. The product was purified by column chromatography on neutral alumina to yield a colourless oil, Rf=0.4 (2% methanol/ CH_2Cl_2). m/e (DCI, ethanol) 563 ($\text{M}^+ + 1$). $\delta_{\text{H}}(\text{CDCl}_3$, 298K) 1.25 (1H, t + t, CH_3), 1.28-1.69 (6H, mult., $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCO}$), 2.76-3.02 (12H, mult., CH_2N ring), 3.22-3.54 (7H, mult., $\text{CH}_2\text{NHCO} + \text{CHCO} + \text{CH}_2\text{CO}$), 4.13 (6H, q, CH_2O), 6.67 (1H, brs, NHCO), 7.40 (3H, mult. arom CH), 7.79 (12H, brd, ortho CH).

50 Example 6

N, N'-bis(2-(N-(3-maleimidopropanoyl)-4-aminobutyl)carboxymethyl)-N''-carboxymethyl-1,4,7-triazacyclononane (10mg) was prepared from the compound of Example 4 (38mg) using a similar procedure to that described for the preparation of the compound of Example 3 from the compound of Example 1.

m/e (FAB, p-NBA matrix) 748 ($\text{M}^+ + 1$) 596; $\delta_{\text{H}}(\text{D}_2\text{O})$ 6.85 (4H, s, $\text{CH}=\text{CH}$) 3.96 (4H, m, CH_2CO_2) 3.79 (8H, m, CH_2NCO and CH_2NHCO) 3.10-3.25 (12H, m, CH_2N) 2.49 (4H, t, $\text{J}=6.3\text{Hz}$, CH_2CONH) 1.82 (3H, m, CH_2C) 1.46 (3H, m CH_2C).

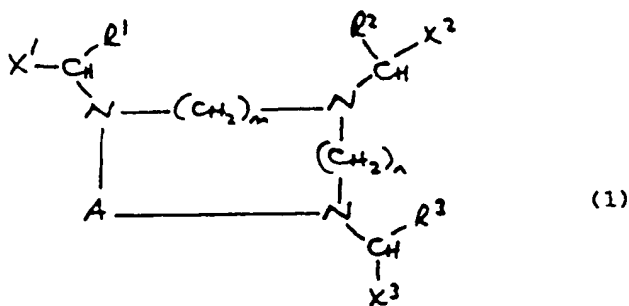
Example 7

N-(2-(N-(3-maleimidopropanoyl)-4-aminobutyl)carboxymethyl)-N', N''-bis(carboxymethyl)-1, 4, 7-triazacyclononane, was prepared from the compound of Example 5 using a similar procedure to that described for the preparation of the compound of Example 3 from the compound of Example 1.

m/e (FAB, p-NBA matrix) 526(M⁺+1) 453, 391, 307; δ_H (D₂O) 6.85 (2H, s, CH=CH) 3.95 (5H, m, CH₂CO₂) 3.79 (4H, m, CH₂NCO and CH₂NHCO) 3.00-3.50 (12H, m, CH₂N) 2.49 (2H, t, J 6.2 Hz, CH₂CONH) 1.69 (3H, m, CH₂-C) 1.46 (3H, m, CH₂-C).

Claims

1. A compound of formula (1):



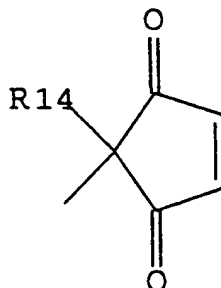
wherein

A is a group $-(CH_2)_p-$ or $-(CH_2)_p N(R)(CH_2)_q-$ where

R is a group $-CH(R^4)X^4$;

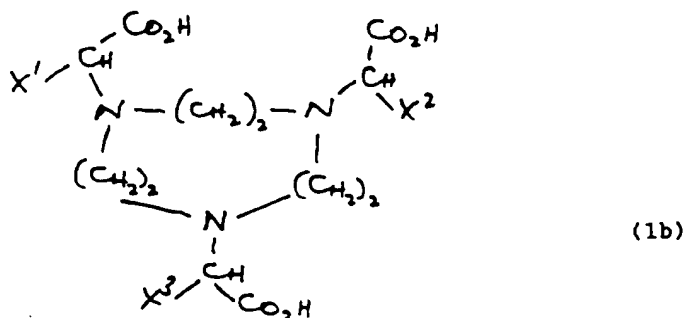
m, n, p, and q is each an integer ≥ 2 ;

R¹, R², R³ and R⁴, which may be the same or different, is each a group $-CO_2H$, $-P(R^5)O_2H$ (where R⁵ is a hydrogen atom or a C₁₋₈ alkyl or alkoxy group), $-PO_3H_2$ or $-CONR^6R^7$ (where R⁶ and R⁷, which may be the same or different is each a hydrogen atom or a C₁₋₈ alkyl group); X¹, X², X³ and X⁴, which may be the same or different, is each a hydrogen atom or a C₁₋₈ alkyl group, or a linker group of the formula $-L-Z$ wherein L is an optionally substituted straight or branched C₁₋₂₀ alkylene, C₂₋₂₀ alkenylene, or C₂₋₂₀ alkynylene chain, optionally interrupted by one or more -O- or -S- atoms or C₅₋₈ cycloalkylene, C₆₋₁₂ aromatic, C₅₋₁₀ heteroaromatic, N(R⁸)-, $-CON(R^8)-$ or $-N(R^8)CO-$ groups (where R⁸ is a hydrogen atom or a C₁₋₆ alkyl group), and Z is a halogen atom or a group selected from OH, -SH, -NH₂, hydrazine (-NHNH₂), $-N(CH_3)NH_2$, $-NHCONHNH_2$, $-NHCSNHNH_2$, or phenylhydrazine, -NCO, -NCS, -COR¹³, [where R¹³ is a halogen atom or a N₃, C₁₋₆ alkoxy, C₆₋₁₂ aryloxy, imidyloxy or imidazolyloxy group], imide, a vinyl group of formula $-Het^1-C(Het^2)=CH_2$ (where Het¹ and Het², which may be the same or different, is each a nitrogen containing heterocyclic group, or Het¹ is a nitrogen containing heterocyclic group and Het² is a hydrogen atom), or a dione of formula



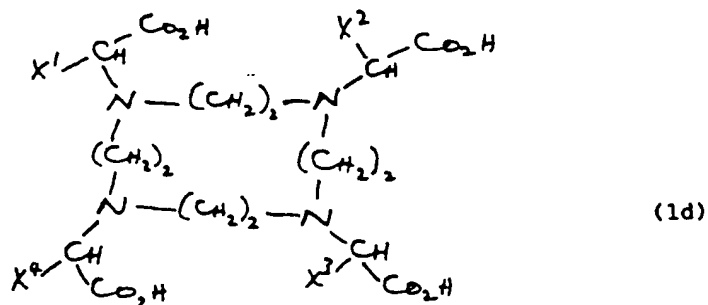
(where R^{14} is a C_{1-4} alkyl group); with the proviso that at least one of X^1 , X^2 , X^3 or X^4 is a linker group; and metal complexes and/or salts thereof.

2. A compound according to Claim 1 wherein one or two of X^1 , X^2 , X^3 and X^4 is a linker group and the remainder are C_{1-8} alkyl groups or hydrogen atoms.
3. A metal complex of a compound of formula (1) as defined in any of the preceding claims in which A is $-(CH_2)_pN(R)(CH_2)_q$, wherein the metal is di- or tripositive and has a coordination number 6 or greater.
4. A metal complex according to Claim 3 wherein the metal is indium, yttrium, gallium, terbium, gadolinium or scandium.
5. A metal complex according to Claim 4 wherein the metal is yttrium.
6. A metal complex of a compound according to Claim 1 or 2 in which A is $-(CH_2)_p$, wherein the metal is di- or tripositive and has a coordination number from 2 up to 6.
7. A metal complex according to Claim 6 wherein the metal is indium, gallium, cobalt or copper.
8. A metal complex according to Claim 7 wherein the metal is indium.
9. A compound of formula (1b) according to claim 1



wherein one or two of X^1 , X^2 or X^3 is a linker group as defined in claim 1 and the remainder are hydrogen atoms; and metal complexes and/or salts thereof.

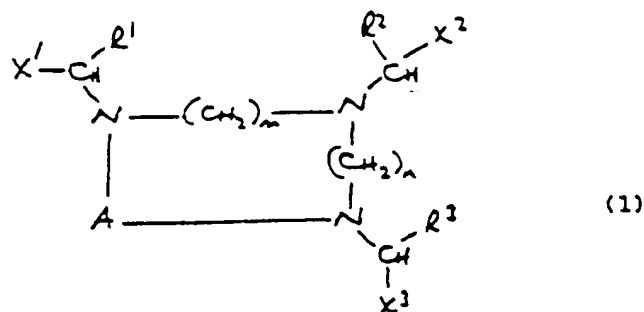
10. A compound of formula (1d) according to claim 1



- wherein one or two of X^1 , X^2 , X^3 or X^4 is a linker group as defined in claim 1 and the remainder are hydrogen atoms; and metal complexes and/or salts thereof.

Patentansprüche

1. Verbindung der Formel (1):

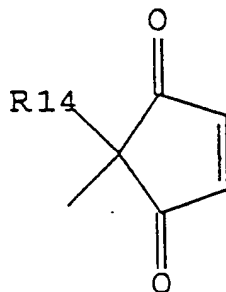


worin

A eine Gruppe $-(CH_2)_p-$ oder $-(CH_2)_pN(R)(CH_2)_q-$ ist, worin R eine Gruppe $-CH(R^4)X^4$ ist;
m, n, p und q jeweils die ganze Zahl 2 sind;

R¹, R², R³ und R⁴, welche gleich oder verschieden sein können, jeweils eine Gruppe $-CO_2H$, $-P(R^5)O_2H$ (worin R⁵ ein Wasserstoffatom oder eine C₁₋₆Alkyl- oder eine Alkoxygruppe ist), $-PO_3H_2$ oder $-CONR^6R^7$ (worin R⁶ und R⁷, welche gleich oder verschieden sein können, jeweils ein Wasserstoffatom oder eine C₁₋₆Alkylgruppe sind), sind;

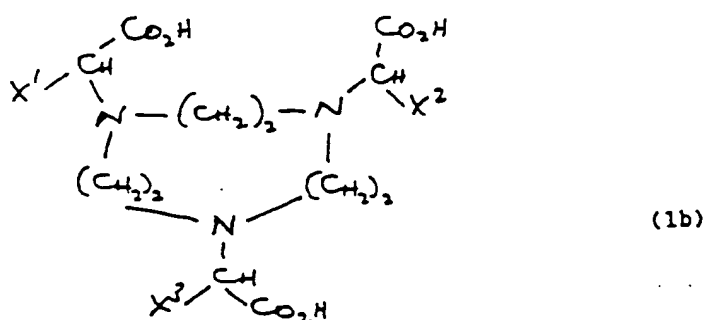
X¹, X², X³ und X⁴, welche gleich oder verschieden sein können, jeweils ein Wasserstoffatom oder eine C₁₋₆Alkylgruppe oder eine Verbindungsgruppe der Formel $-L-Z$ sind, worin L eine gegebenenfalls substituierte, gerade oder verzweigte C₁₋₂₀Alkyl-, C₂₋₂₀Alkenyl- oder C₂₋₂₀Alkynylkette ist, die gegebenenfalls durch ein oder mehrere -O- oder -S- Atome oder C₅₋₈Cycloalkylengruppen, aromatische C₆₋₁₂Gruppen, heteroaromatische C₅₋₁₀Gruppen, N(R⁸)-, $-CON(R^8)-$ oder $-N(R^8)CO-$ Gruppen (worin R⁸ ein Wasserstoff oder eine C₁₋₆Alkylgruppe ist) unterbrochen ist, und Z ist: ein Halogenatom oder eine Gruppe, ausgewählt aus OH, -SH, -NH₂, Hydrazin ($-NHNH_2$), $-N(CH_3)NH_2$, $-NHCONH_2$, $-NHCSNHNH_2$ oder Phenylhydrazin, -NCO, -NCS, $-COR^{13}$, [worin R¹³ ein Halogenatom oder eine N₃-, C₁₋₆Alkoxy, C₆₋₁₂Aryloxy-, Imidyloxy- oder Imidazolyloxygruppe ist], Imid, einer Vinylgruppe der Formel $-Het^1-C(Het^2)=CH_2$ (worin Het¹ und Het², welche gleich oder verschieden sein können, jeweils eine Stickstoff-enthaltende heterocyclische Gruppe sind, oder Het¹ eine Stickstoff-enthaltende heterocyclische Gruppe ist und Het² ein Wasserstoffatom ist), oder ein Dion der Formel



(worin R¹⁴ eine C₁₋₄Alkylgruppe ist); mit der Maßgabe, daß mindestens eine von X¹, X², X³ oder X⁴ eine Verbindungsgruppe ist;
und Metallkomplexe und/oder Salze davon.

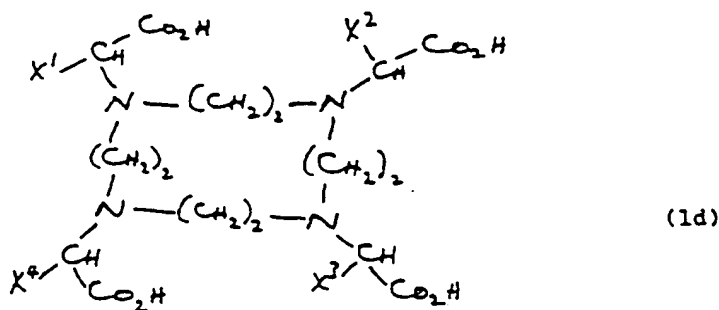
2. Verbindung nach Anspruch 1, wobei eine oder zwei von X¹, X², X³ und X⁴ eine Verbindungsgruppe ist bzw. sind und der Rest C₁₋₆Alkylgruppen oder Wasserstoffatome sind.

3. Metallkomplex einer Verbindung der Formel (1), wie in einem vorhergehenden Anspruch definiert, in welcher A - $(CH_2)_p N(R)(CH_2)_q$ ist, worin das Metall zweifach oder dreifach positiv ist und eine Koordinationszahl von 6 oder höher aufweist.
- 5 4. Metallkomplex nach Anspruch 3, wobei das Metall Indium, Yttrium, Gallium, Terbium, Gadolinium oder Scandium ist.
5. Metallkomplex nach Anspruch 4, wobei das Metall Yttrium ist.
6. Metallkomplex einer Verbindung nach Anspruch 1 oder 2, in welcher A $-(CH_2)_p-$ ist, wobei das Metall zweifach oder dreifach positiv ist und eine Koordinationszahl von 2 bis 6 aufweist.
- 10 7. Metallkomplex nach Anspruch 6, wobei das Metall Indium, Gallium, Cobalt oder Kupfer ist.
8. Metallkomplex nach Anspruch 7, wobei das Metall Indium ist.
- 15 9. Verbindung der Formel (1b) nach Anspruch 1



wobei eine oder zwei von X¹, X² oder X³ eine wie in Anspruch 1 definierte Verbindungsgruppe ist bzw. sind und der Rest Wasserstoffatome sind;
 35 und Metallkomplexe und/oder Salze davon.

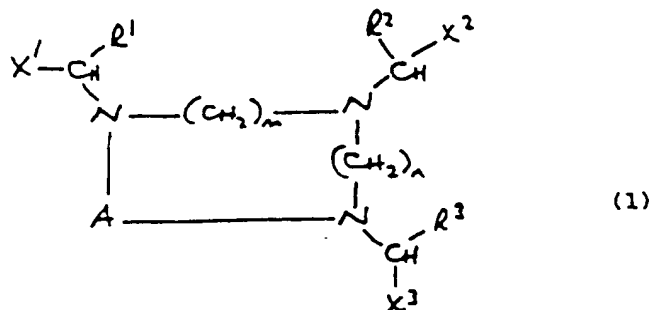
10. Verbindung der Formel (1d) nach Anspruch 1



wobei eine oder zwei von X¹, X², X³ oder X⁴ eine wie in Anspruch 1 definierte Verbindungsgruppe ist bzw. sind und der Rest Wasserstoffatome sind;
 55 und Metallkomplexe und/oder Salze davon.

Revendications

1. Composé de formule (1):



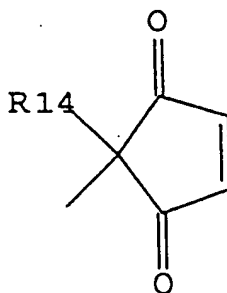
dans laquelle

A est un groupe $-(CH_2)_p-$ ou $-(CH_2)_pN(R)(CH_2)_q-$, où

R est un groupe $-CH(R^4)X^4$;

m, n, p et q sont chacun un nombre entier égal à 2;

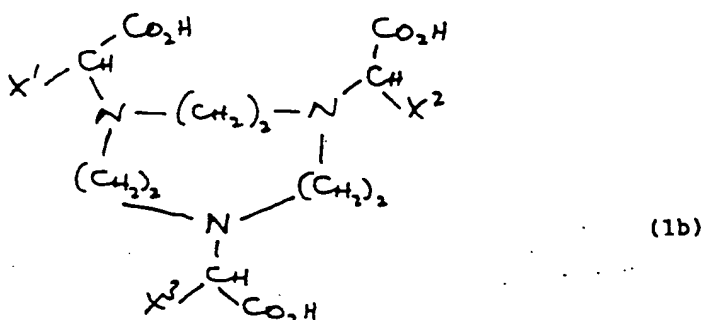
R¹, R², R³ et R⁴, qui peuvent être identiques ou différents, sont chacun un groupe -CO₂H, -P(R⁵)O₂H (dans lequel R⁵ est un atome d'hydrogène ou un groupe alkyle ou alcoxy en C₁-C₆), -PO₃H₂ ou -CONR⁶R⁷ (où R⁶ et R⁷, qui peuvent être identiques ou différents, sont chacun un atome d'hydrogène ou un groupe alkyle en C₁-C₆); X¹, X², X³ et X⁴, qui peuvent être identiques ou différents, sont chacun un atome d'hydrogène ou un groupe alkyle en C₁-C₆, ou un groupe de liaison de formule -L-Z, dans laquelle L est une chaîne alkylène en C₁-C₂₀, alcénylène en C₂-C₂₀ ou alcynylène en C₂-C₂₀, linéaire ou ramifiée, éventuellement substituée, éventuellement interrompue par un ou plusieurs atomes -O- ou -S-, ou des groupes cycloalkylène en C₅-C₈, aromatiques en C₆-C₁₂, hétéroaromatiques en C₅-C₁₀, N(R⁸)-, -CON(R⁸)- ou -N-(R⁸)CO- (où R⁸ est un atome d'hydrogène ou un groupe alkyle en C₁-C₆), et Z est un atome d'halogène ou un groupe choisi parmi OH, -SH, -NH₂, hydrazine (-NHNH₂), -N(CH₃)NH₂, -NHCONHNH₂, -NHCSNHNH₂ ou phénylhydrazine, -NCO, -NCS, -COR¹³ [dans lequel R¹³ est un atome d'halogène ou un groupe N₃, alcoxy en C₁-C₆, aryloxy en C₆-C₁₂, imidyloxy ou imidazyloxy], imide, un groupe vinyloxy de formule -Het¹-C(Het²)=CH₂ (dans laquelle Het¹ et Het², qui peuvent être identiques ou différents, sont chacun un groupe hétérocyclique azoté, ou bien Het¹ est un groupe hétérocyclique azoté et Het² est un atome d'hydrogène), ou une dione de formule



(dans laquelle R¹⁴ est un groupe alkyle en C₁-C₄); à condition que l'un au moins des radicaux X¹, X², X³ et X⁴ soit un groupe de liaison; et ses sels et/ou complexes métalliques.

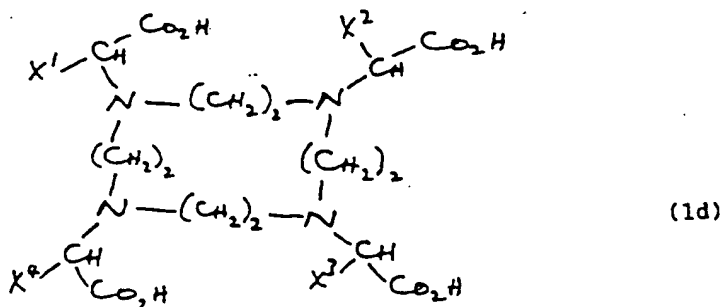
2. Composé selon la revendication 1, dans lequel un ou deux des radicaux X¹, X², X³ et X⁴ est un groupe de liaison et ceux qui restent sont des groupes alkyle en C₁-C₆ ou des atomes d'hydrogène.
3. Complexe métallique d'un composé de formule (1) telle que définie dans l'une quelconque des revendications précédentes, dans lequel A est -(CH₂)_pN(R)(CH₂)_q-, où le métal porte deux ou trois charges positives et possède un indice de coordination égal ou supérieur à 6.

4. Complexe métallique selon la revendication 3, dans lequel le métal est l'indium, l'yttrium, le gallium, le terbium, le gadolinium ou le scandium.
5. Complexe métallique selon la revendication 4, dans lequel le métal est l'yttrium.
6. Complexe métallique d'un composé selon la revendication 1 ou 2, dans lequel A est $-(CH_2)_p-$, où le métal porte deux ou trois charges positives et possède un indice de coordination de 2 à 6.
7. Complexe métallique selon la revendication 6, dans lequel le métal est l'indium, le gallium, le cobalt ou le cuivre.
8. Complexe métallique selon la revendication 7, dans lequel le métal est l'indium.
9. Composé de formule (1b) selon la revendication 1



dans lequel un ou deux des radicaux X^1 , X^2 et X^3 est un groupe de liaison tel que défini dans la revendication 1, et ceux qui restent sont des atomes d'hydrogène; et ses sels et/ou complexes métalliques.

10. Composé de formule (1d) selon la revendication 1



dans lequel un ou deux des radicaux X^1 , X^2 , X^3 et X^4 est un groupe de liaison tel que défini dans la revendication 1, et ceux qui restent sont des atomes d'hydrogène; et ses sels et/ou complexes métalliques.